Agar Diffusion Assay

Introduction

The agar diffusion assay is used to check the sensitivity of bacteria to specific substances, for instance, AHL we used in the experiment. We can check the situation that how bacteria react to those particles, for example, observe the fluorescent of bacteria, then measure the diameter of the circle that is composed of glowing bacteria. This technique allows us to check whether some mechanism of bacteria can be activated by the signal we provide with.

Reference:
1.Ravn, L., Christensen, A. B., Molin, S., Givskov, M., & Gram, L. (2001). Methods for detecting acylated homoserine lactones produced by Gram-negative bacteria and their application in studies of AHL-production kinetics. *Journal of Microbiological Methods*, *44*(3), 239-251.
2.Khan, Z. A., Siddiqui, M. F., & Park, S. (2019). Current and emerging methods of antibiotic susceptibility testing. *Diagnostics*, *9*(2), 49.

Materials

- 1.5% LB agar plate
- 0.3% Soft agar
- toothpick

Procedure

Operation

1. Heat up soft agar utill it fully melts
2. Add the bacteria we want to test in the soft agar, than mix them by vibration
3. Evenly pure the soft agar on the LB plate
4. When soft agar is solidified, use the big end of 1ml tip to press the agar plate
5. Use toothpick to remove the region we just pressed
6. Add the solution we want to test in the hole we made in step 5
7. Put the plate in 37◦ C environment